The interaction of Moringa Oleifera with Chromium and the effect on Chinese Hamster Ovary cell toxicity

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Abstract

Moringa oleifera is a plant with countless nutritional and therapeutic properties, and it is considered as one of the supplements that prevent diseases because of its antioxidant effects. In this study, treatment of CHO (Chinese Hamster Ovary) cells with commercially available *M. oleifera* (900 µg/ml, 4 mg/ml, 9 mg/ml, and 17.5 mg/ml) induced cytotoxic effects. Furthermore, cells were treated with different concentrations of chromium (0.25, 0.5, and 1 µM) combined with *M. oleifera* extract to evaluate its protective effect against heavy metals. Statistical analyses indicated that *M. oleifera* extract is toxic to the cells in a dose-dependent manner. Therefore, our results suggest that chromium reduces the toxicity of *M. oleifera* by increasing cell viability. These observations suggest that chromium may antagonize the toxicity *M. oleifera* extracts and acts as a protective agent to cell toxicity.

Introduction

Chromium is a naturally occurring element found in rocks and soil which is taken up by plants and entering the food chain for human consumption. It is an essential micronutrient that can regulate lipid and carbohydrate metabolism in animals. Studies have found organisms with chromium deficiency develops metabolic disorders. Diabetic patients can benefit from consuming supplements containing Cr(III) control lipids, blood glucose, and insulin concentration. However, epidemiological studies indicate that chromium containing compounds are mutagenic and carcinogenic agents in animals and cell culture models. *M. oleifera* tree is a multipurpose plant because on its leaves, flowers, seeds, roots, and trunk are edible. Furthermore, *M. oleifera* plant has been studied due to its anti-tumor, anti-cancer, and anti-oxidant capabilities for medical use. Research is in progress in developing new drugs using the leaf part of the plant, due to *M. oleifera* having antioxidant, antimicrobial, and anticancer properties. The leaves and seeds have the ability for preventing the harmful effects of oxidative stress by scavenging free radicals. Yet, no studies have evaluated the interaction of *M. oleifera* extract and heavy metals. Therefore, the purpose of this study is to use commercially available *M. oleifera* extract to study the cytotoxicity on CHO cells and observe if there is any protective effect of *M. oleifera* against chromium toxicity.

Method & Materials

Cell Culture and Seeding

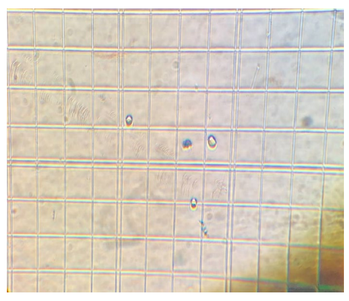
Chinese hamster ovary AA8 (CHO) cells were culture in T25 cell culture flask with Basal Medium Eagle (DMEM) and cells were stored in a 37°C, 5% CO2/air incubator.

Cells were seeded into a 6 well tissue culture plate 20 mm glass-bottom dish at 120,000 cells and cell culture plates were incubated at 37°C for 24 hours.

Treatment

All reagents, chemicals, and extracts were prepared to their separate concentrations the same day of the cell's treatment.

Different doses of *Moringa oleifera* extract (9 mg/ml, 4 mg/ml, 900 ug/ml) and $Na2CrO_4$ (1 µM, 0.5 µM, and 0.25 µM) added to each dish and controls was left alone. Cultures were treated in combination with M. oleifera and chromium. . The cells were treated with agents, allowing 24 h chemical exposure. Cells were then collected by trypsinization and stained with trypan blue to determine cell viability.



Trypan Blue Assay of Cell Viability
In the protocol presented here, The cells were counted; nonviable cells stain blue, and viable cells remain opaque. Its principle suggest that live cells possess intact cell membrane that excludes trypan blue dye, whereas dead cells do not.

Conclusion

CHO AA8 cell were treated with *M. oleifera* extract, 900 µg/ml, 4 mg/ml, 9mg/ml, and 17.5 mg/ml, indicated a low to high cell toxicity in a dose-dependent fashion. At 17.5 mg/ml is the recommended dose by the supplier of the commercially available *M. oleifera* extract, it was found to be toxic to the cells. For the combined treatment of *M. oleifera* with chromium, we observed that cell viability improves. Despite our expectations that *M. oleifera* to have a protective effect on chromium toxicity, our results indicate that chromium has a protective effect against *M. oleifera* toxicity.

Results & Discussion

Table 1. Results of Cytotoxicity of *M. oleifera* extract, Chromium, and Mo*Cr

Treatment	Mo extract (mg)	Cr (µM)	trial 1	trial 2	trial 3	Mean ± SD)
Moringa oleifera	0	0	97.33	98	97	95.11±1.50
	0.9	0	89.95	84.31	87.74	87.33±2.32
	4	0	57.69	57.78	52	55.82±2.70
	9	0	27.27	34.1	34	31.79±3.2
	17.5	0	23.08	25	33.33	27.1±4.45
Chromium						
	0	0	95.45	97.3	97.06	96.6±1.01
	0	0.25	90.4	90.9	91.3	90.9±0.5
	0	0.5	85	87.5	80.49	84.3±3.55
	0	0	98.33	95.24	97.22	95.3±1.95
	0	1	60	58.3	64	60.8±2.93
Cr + Mo						
	0	0	95.45	97.3	97.06	96.6±1.01
	0.9	0.25	91.3	90.9	92.86	91.7±1.04
	0.9	0.5	80.8	77.7	82	80.2±2.22
	0	0	98.33	95.24	97.22	95.3±1.95
	0.9	1	65.38	73.2	65.6	68.1±4.45
	9	1	52.17	58.8	55.6	55.5±3.32
	17.5	1	45.54	52.6	50.2	50±1.95

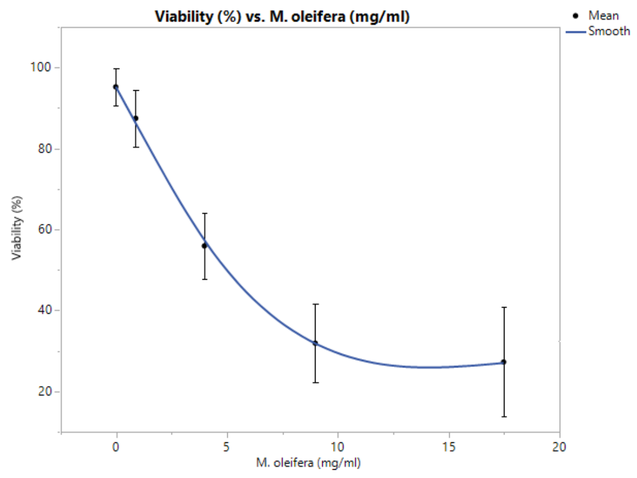


Fig 1. Cell viability analyses by trypan blue assay in CHO cells. The cells were treated with *M. oleifera* with different concentrations (0 mg 900 µg/ml, 4 mg/ml, 9 mg/ml, and 17.5 mg/ml). The control (0 mg) was incubated at the same time as the treated cells. Data are described as mean ± standard deviation of three different experiments.

Table 2. Results of Regression analysis on *Moringa oleifera* extract (mg/ml)

Regression analysis	
p value	<.001
R^2	0.986
Equation	$y=95.24 - 10.77*X + 0.394*X^2$

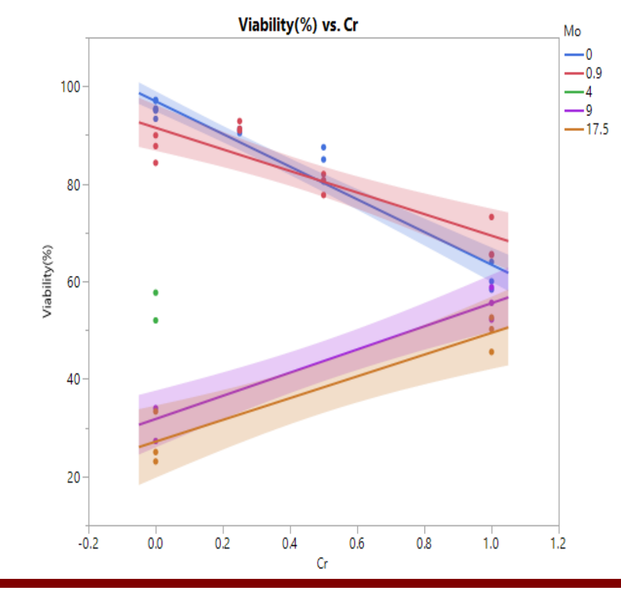


Fig 2. Data shows a scatterplot of the relationship between cell viability (%) and Chromium in different concentrations of *M. oleifera* (mg/ml).

Table 5. Results of ANCOVA analysis on *M. oleifera* extract, Chromium, and Mo*Cr

Source	Df	SS	F	p-value
Mo	1	16633.04	183.39	p<.0001
Cr	1	805.65	8.88	p=0.0048
Mo*Cr	1	4031.06	44.44	p<.0001
R Squared =0.841				
Equation				
Y = (0) =96.93 - 33.51*Cr				
Y = (0.9) =91.5 - 22.14*Cr				
Y (4) = 55.82				
Y (9) = 31.79 + 23.73*Cr				
Y (17.5) = 27.14 + 22.31*Cr				

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Reference

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